Real-time geographic settling of a hybrid zone between the invasive winter moth (Operophtera brumata L.) and the native Bruce spanworm (O. bruceata Hulst)

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Abstract

Hybridization plays an important and underappreciated role in shaping the evolutionary trajectories of species. Following the introduction of a non-native organism to a novel habitat, hybridization with a native congener may affect the probability of establishment of the introduced species. In most documented cases of hybridization between a native and a non-native species, a mosaic hybrid zone is formed, with hybridization occurring heterogeneously across the landscape. In contrast, most naturally occurring hybrid zones are clinal in structure. Here we report on a long-term microsatellite dataset that monitored hybridization between the invasive winter moth, Operophtera brumata (Lepidoptera: Geometridae), and the native Bruce spanworm, O. bruceata, over a 12-year period. Our results document one of the first examples of the real-time formation and geographic settling of a clinal hybrid zone. In addition, by comparing one transect in Massachusetts where extreme winter cold temperatures have been hypothesized to restrict the distribution of winter moth, and one in coastal Connecticut, where winter temperatures are moderated by Long Island Sound, we find that the location of the hybrid zone appears to be independent of environmental variables and maintained under a tension model wherein the stability of the hybrid zone is constrained by population density, reduced hybrid fitness, and low dispersal rates. Documenting the formation of a contemporary clinal hybrid zone may provide important insights into the factors that shaped other well-established hybrid zones.

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Short Title: Winter moth hybrid zone formation

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Abstract:

Hybridization plays an important and underappreciated role in shaping the evolutionary trajectories of species. Following the introduction of a non-native organism to a novel habitat, hybridization with a native congener may affect the probability of establishment of the introduced species. In most documented cases of hybridization between a native and a non-native species, a mosaic hybrid zone is formed, with hybridization occurring heterogeneously across the landscape. In contrast, most naturally occurring hybrid zones are clinal in structure. Here we report on a long-term microsatellite dataset that monitored hybridization between the invasive winter moth, *Operophtera brumata*(Lepidoptera: Geometridae), and the native Bruce spanworm, *O. bruceata*, over a 12-year period. Our results document one of the first examples of the real-time formation and geographic settling of a clinal hybrid zone. In addition, by comparing one transect in Massachusetts where extreme winter cold temperatures have been hypothesized to restrict the distribution of winter moth, and one in coastal Connecticut, where winter temperatures are moderated by Long Island Sound, we find that the location of the hybrid zone appears to be independent of environmental variables and maintained under a tension model wherein the stability of the hybrid zone is constrained by population density, reduced hybrid fitness, and low dispersal rates. Documenting the formation of a contemporary clinal hybrid zone may provide important insights into the factors that shaped other well-established hybrid zones.

Keywords: Tension zone, hybrid fitness, introgression, Lepidoptera, forest pest.

Introduction:

Hybridization is a driver of speciation and evolutionary trajectories across the tree of life (Allendorf, Leary, Spruell, & Wenburg, 2001; Costedoat, Pech, Chappaz, & Gilles, 2007; Harrison & Larson, 2014; Mallet, 2005). While numerous pre- and post-zygotic barriers exist in most natural ecosystems to reduce genetic exchanges between species, human-mediated disturbance and climate change have led to increased hybridization rates across a diversity of taxonomic groups (Gomez, Gonzalez-Megias, Lorite, Abdelaziz, & Perfectti, 2015; Hegarty, 2012; Larson, Tinghitella, & Taylor, 2019). The accidental introduction of non-native organisms to novel habitats has further increased these rates by uniting previously disjunct species or genetically distinct populations (Chown et al., 2015; Havill et al., 2012; Havill et al., 2021; Michaelides, While, Bell, & Uller, 2013). In addition to illuminating factors that may be important in invasion ecology, studying the real-time formation of hybrid zones between native and non-native species may provide a type of natural laboratory, providing important insights into how other well-established hybrid zones may have formed and settled over evolutionary timescales. As such, recent work has highlighted the importance of studying newly-formed hybrid zones for understanding speciation and the preservation of species boundaries (Johannesson, Le Moan, Perini, & Andre, 2020; Larson et al., 2019).

These natural laboratories are particularly important because most documented hybrid zones have likely existed for thousands of years and formed following the movement of species in response to long-term processes such as changing climates during the Quaternary climatic oscillations (e.g., Ryan et al., 2018; Ryan et al., 2017; Scriber, 2011; Taylor, Larson, & Harrison, 2015). Natural hybrid zones frequently have a clinal structure, with a narrow, linear geographic zone of admixture where phenotypic and genetic states change across a gradient between parent species (Barton & Hewitt, 1985; Endler, 1977). In contrast, most documented hybrid zones created in contemporary settings between introduced and native species have a mosaic structure (see Harrison & Rand, 1989), with zones of genetic exchange spread across the landscape in a patchy and non-linear fashion (e.g. Cordeiro et al., 2020; Havill et al., 2012). Therefore, additional examples of newly formed clinal hybrid zones are needed to better understand the evolutionary and ecological processes that shape these temporally and spatially dynamic regions of secondary contact.

Species of moths and butterflies (Insecta: Lepidoptera) have provided some of the most stunning examples of the diversity of interactions resulting from hybridization (e.g., Ipekdal, Burban, Saune, Battisti, & Kerdelhue, 2020; Lucek, Butlin, & Patsiou, 2020; Ryan et al., 2018; Ryan et al., 2017; Scriber, 2011). Here we explore the formation of a hybrid zone in northeastern North America between the introduced European winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae) and the native Bruce spanworm, *O. bruceata* Hulst. Winter moth is native to western Eurasia and North Africa (Ferguson, 1978) and originally became established in North America in Nova Scotia in the 1930s, where it was identified as a major pest in

apple orchards and oak-dominated hardwood forests (Embree, 1966, 1967). Subsequently, populations were identified in Oregon as a pest in hazelnut (filbert) orchards in the 1950s (Kimberling, Miller, & Penrose, 1986), British Columbia as a pest in apple orchards and of urban trees in the 1970s (Gillespie, Wratten, Cruickshank, Wiseman, & Gibbs, 1978), and most recently in the northeastern United States (hereafter, the "Northeast") as a pest of blueberries, cranberries, and many native deciduous trees in the 1990's (Elkinton et al., 2010; Elkinton, Liebhold, Boettner, & Sremac, 2014). Each of these regions were likely the result of independent invasions from Europe (Andersen, Havill, Caccone, & Elkinton, 2021), and while successful biological control programs have reduced the abundance and economic impacts of this important pest in each invaded region (Elkinton, Boettner, Liebhold, & Gwiazdowski, 2015; Elkinton, Boettner, & Broadley, 2021; Kimberling et al., 1986; Roland & Embree, 1995), populations of winter moth continue to persist at low densities in each location. Previous work in this system has shown that winter moth and Bruce spanworm hybridize readily in the field (Andersen et al., 2019; Elkinton et al., 2010, 2014; Havill et al., 2017). Additionally, in the Northeast it has been documented that the proportions of individuals of winter moth versus Bruce spanworm can be modeled using logistic regression, with populations proximate to Boston, Massachusetts being nearly 100% winter moth, and populations in western Massachusetts being nearly 100% Bruce spanworm (Elkinton et al., 2014). This gradient in winter moth and Bruce spanworm population densities in the Northeast therefore raises the possibility that a clinal hybrid zone may exist in this region, making it one of the first documented cases of this type of hybrid zone between an introduced and a native species.

We explored the spatial and temporal dynamics of the hybrid zone between the invasive winter moth and native Bruce spanworm by collecting moths with pheromone traps along two transects that crossed the leading edge of winter moth spread in the Northeast region. One of these transects was located along Route 2 in Massachusetts (hereafter the "Massachusetts transect") and was sampled over a 12-year period (from 2007 to 2018) where a gradient across decreasing extreme cold winter temperatures has been hypothesized to limit the distribution of winter moth (Elkinton, Lance, Boettner, Khrimian, & Leva, 2011). The second transect is located along the coast of southern Connecticut following Route 1 (hereafter the "Connecticut transect") and was sampled over a 3-year period (2016-2018). This second transect was added so that we could compare the role of winter temperatures on the geographic location of the hybrid zone as temperatures along the Connecticut transect are milder than at any point along the Massachusetts transect, and geographic settling would therefore be independent of low winter temperatures. With these data, we explore: 1) the structure and movement of the hybrid zone in the Northeast, 2) changes in the rate of hybridization over time, and 3) the impacts of environmental gradients and population densities in the regulation of the hybrid zone.

Methods and Materials:

Pheromone surveys

In Massachusetts, for each year from 2007-2018, adult male moths were collected using pheromone-baited lures (Great Lakes IPM, Vestaburg, MI; Elkinton et al., 2010, 2011) hung in plastic bucket traps (Gempler's Inc., Janesville, WI) at points along the Massachusetts transect (Figure 1). These included 49 unique localities; however, due to factors such as road construction, early-season snow, ice storms, vandalism, and mouse predation, an average of 22 trap localities ($\pm$ 1.5) were sampled each year, with a minimum of 16 traps in 2015 and a maximum of 32 traps in 2017 and 2018. In addition, after 2009, we discontinued monitoring traps close to the Boston Metropolitan area (T21-T25) in an effort to conserve survey resources because these traps were entirely composed of winter moth individuals (Elkinton et al., 2014). From 2007-2016, moths were collected from traps twice during the sampling period (late November through early January), coinciding with observations of male moth flight. In 2017, moths were collected during the same period weekly, and in 2018, traps were sampled bi-weekly. For the Connecticut transect (Figure 1), pheromone traps were deployed from 2016-2018. These included 19 localities in 2016, though due to factors mentioned above, in 2017 and 2018, only 18 and 17 of these localities were sampled, respectively. Along this transect, moths were collected weekly from late November through early January. A list of trap locations is provided in Supplemental Table S1. Upon collection, moths were removed from traps, placed in glassine envelopes (Uline Inc., Pleasant

Prairie, WI), and stored at -80°C. The total number of moths collected over the flight period in each trap was counted to provide an estimate of relative population density surrounding each trap location.

DNA extraction and microsatellite genotyping

For the Massachusetts transect from 2007-2016, up to 20 moths were selected haphazardly from each trap's pooled sampling (i.e., from combining the contents of the two collection events). For the Massachusetts transect traps from 2017 and 2018, and from all Connecticut transect traps, 20 moths were selected haphazardly from each collection event (i.e., each weekly or bi-weekly collection event was processed independently). Prior to the isolation of genomic DNA, the wings and uncus were preserved as morphological vouchers as these contain characters that may be useful for species identification (Griffin, Chandler, Andersen, Havill, & Elkinton, 2020; Troubridge and. Fitzpatrick 1993). The remaining body parts were placed in a 2.0 ml micro-centrifuge tube (USA Scientific Inc., Ocala, FL) with 100 µl of PBS buffer (Sigma Aldrich, St. Louis, MO) and homogenized with a sterile 3/16" stainless steel bead (GlenMills Inc., Clifton, NJ), using a FastPrep-24 Sample Homogenizer (MP Biomedicals, Santa Ana, CA). DNA was extracted and purified using the EZNA® Tissue DNA extraction kit (Omega Bio-tek; Norcross, GA), following the manufacturer protocols.

From each sample, 11 polymorphic microsatellite loci were amplified following Havill et al. (2017), and run with the GeneScan 500 LIZ size standard (Thermo Fisher Scientific; Waltham, MA), on a Thermo Fisher Scientific 3730xl DNA Analyzer at the DNA Analysis Facility on Science Hill at Yale University. Fragment lengths were scored in the software GENEIOUS v. R11 (https://www.geneious.com), using the microsatellite plugin.

Classification of samples to species and hybrid categories

The probability of assignment (Z) of each sample as a pure Bruce spanworm, a pure winter moth, or a hybrid (either F1, F2, Bruce spanworm-backcross, or winter moth-backcross) was estimated using the Bayesian-assignment program NewHybrids v 1.1 b3 (Anderson, 2008; Anderson & Thompson, 2002). We used uniform priors, random starting seeds, burn-in periods of 100,000 generations, and a post-burn-in runtime of 1,000,000 generations. A separate dataset was run for each transect and year combination to reduce assignment errors, given that individuals from one year could be the offspring of individuals from the previous year. Datasets were then filtered so that only individuals with [?] 10 successfully scored loci were included. Four independent runs were performed for each dataset, and the assignment scores were then averaged across runs. We interpreted samples with Z [?] 0.75 to any one category as obtaining "strong support", and samples with 0.5 [?] $Z < 0.75$ as obtaining "moderate support". If a sample was not assigned to any category with Z [?] 0.5, it was classified as having "weak support" to the category with the highest Z score.

To determine whether there was temporal or spatial variation in hybridization rates across each transect, the mean proportions of hybrids were calculated by dividing the number of genotyped individuals from each trap classified to one of the four hybrid categories by NewHybrids (as described above). Differences between years and between traps were compared using an analysis of variance (ANOVA) as implemented in R v 4.0.2 (R Core Team, 2020).

Hybrid zone movement

For each transect-year we fit hybrid zone equilibrium cline models using population ancestry proportions to estimate the shape and center of the hybrid zone. First, for each transect, the distance (in km) of each trap from the respective westernmost trap for that transect (trap T00 [42.6714deg N, 73.0145deg W] for the Massachusetts transect, and trap CT01 [41.0798deg N, 73.7054deg W] for the Connecticut transect), was calculated using the Latitude/Longitude Distance Calculator available at (https://www.nhc.noaa.gov/gccalc.shtml). Then, for each transect-year, the population coefficient of assignment (Q) to Bruce spanworm at each trap was estimated using the software program Structure v.2.3.2 (Falush, Stephens, & Pritchard 2003; Pritchard, Stephens, & Donnelly, 2000) based on two population (K= 2) analyses using the admixture model, correlated allele frequencies, and default settings, with random start-

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ing values, runtimes of 200,000 generations, and burn-in periods of 20,000 generations, for each year-transect combination. Finally, using the R package 'hzar' v 0.2-5 (Derryberry, Derryberry, Maley & Brumfield, 2014), a null model, plus three different hybrid-zone models: 1) minimum and maximum frequencies fixed to 0 and 1, and with no exponential decay tails, 2) minimum and maximum frequencies as free parameters, with no exponential decay tails, and 3) minimum and maximum frequencies as free parameters, with both tails as independent parameters, were used to estimate the center of the hybrid zone based on three independent analyses with chain lengths of 100,000 generations, burn-in periods of 10,000 generations, using random starting variables for each analysis. The maximum likelihood values for each run were then compared to determine which model provided the best fit to the observed dataset, from which the center of the hybrid zone and the hybrid cline was estimated for each transect-year combination.

Comparison of minimum winter temperatures

To document differences in minimum winter temperatures along the Massachusetts and Connecticut transects, daily minimum temperatures were extracted from PRISM daily minimum temperature rasters available from the PRISM Climate Group at http://prism.oregonstate.edu in ArcMap v. 10.7.1 (Esri Inc., West Redlands, CA) for each trap location along both transects from December 1st through March 31st from 2007-2018. At each trap, these results were then averaged to produce a mean of the yearly minimum temperatures across the twelve-year period. To determine whether there were differences between trap localities within a transect, and globally between the two transects, linear regression analyses were performed with the mean of the minimum temperatures as the response variable and distance and state as predictor variables in R.

Results:

DNA extraction and microsatellite genotyping

After filtering, 9,983 adult moths were included in the analyses. This included an average of 367.1 +- 14.81 moths per year from 2007-2016 plus 1,369 and 811 moths in 2017 and 2018, respectively, for the Massachusetts transect, and 1,374.3 +- 123.7 moths per year from 2016-2018 for the Connecticut transect.

Classification of samples to species and hybrid categories

Across both transects, 4,026 moths were classified as winter moth ($n = 4,026$ with strong support, and $n = 1$ with moderate support), 5,434 as Bruce spanworm ($n = 5,432$ with strong support, $n = 1$ with moderate support, and $n = 1$ with weak support), and 523 as one of the four hybrid-categories with the majority ($n = 281$) being classified as F1 hybrids ($n = 254$ with strong support, $n = 23$ with moderate support, and $n = 4$ with weak support), 155 individuals as F2 hybrids ($n = 125$ with strong support, $n = 23$ with moderate support, and $n = 7$ with weak support), 86 individuals as winter moth-backcrosses ($n = 60$ with strong support, $n = 18$ with moderate support, and $n = 8$ with weak support) and 1 individual as a Bruce spanworm-backcross (with moderate support). The number of moths classified to each hybrid category along the Massachusetts transect is presented in Table 1 and along the Connecticut transect in Table 2. The proportions of individuals classified as winter moth, Bruce spanworm, or to one of the four hybrid classes are presented in Figures 2 and 3a for the Massachusetts and Connecticut transects, respectively. The numbers of individuals assigned to each hybrid classification are presented in Figures 4 and 3b for the Massachusetts and Connecticut transects, respectively.

On average, we observed a hybridization rate of 5.67% +- 0.62% across years along the Massachusetts transect. We found no significant difference in hybridization rates among years along the Massachusetts transect (P=0.332, F=1.139, df=11), however, we did observe a significant difference in mean hybridization rates among traps for all years combined (P=0.0024, F=1.98, df=32), though none of the pairwise comparisons among traps were significant based on Tukey's HSD test (Figure 5). Along the Connecticut transect, we observed a hybridization rate of 5.97% +- 0.91% across years, with significant differences in hybridization rates between years (P=0.0285, F=3.817, df=2), with the hybridization rates between the 2017 and 2018 transect surveys being significantly different based on Tukey's HSD test (adjusted P= 0.027). As per the Massachusetts transect, significant differences were also observed among traps for all years combined

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(P=0.0082, F=2.569, df=18), though none of the pairwise comparisons were significant based on Tukey's HSD test (Figure 6).

Hybrid zone movement

For eight of the transect-years, the best fit of was the model with free minimum and maximum frequencies and no tails, for six transect-years it was the model with fixed frequencies and no tails, and for one transect-year it was the model with free frequencies with tails (Table 3; Supplementary Figures S1 and S2). For the Massachusetts transect, the analyses for 2009, 2011, and 2012 resulted in cline shapes with minimum or maximum frequencies that did not approach 0 and 1 (Supplemental Figure S2). This result was likely due to the fact that for those years, we did not sample far enough into the Bruce spanworm (2009) or winter moth (2011 and 2012) dominated areas. Consequently, these years were left out of Figure 7 as the centers could not be accurately estimated (Table 3). Cline analyses of the hybrid zones along the Massachusetts and Connecticut transects indicated that the center of the hybrid zones moved westward during our study period (Figures 7 and 8). Along the Massachusetts transect, in 2007 the center of the hybrid zone was located approximately 131 km from trap T00, and by 2018 the center of the hybrid zone was located approximately 103 km from trap T00 (Table 3 and Figure 7). During our survey period, the location of the center of the hybrid zone moved 3.1 km from 2007 to 2008, then moved more rapidly from 2008 to 2010 (6.2 km/yr), then moved steadily westward from 2014-2017 (2.4 km/yr), before retreating eastward 7.7 km in 2018. Along the Connecticut transect, in 2016 the center of the hybrid zone was located approximately 109 km from trap CT01, in 2017 it moved approximately 8.9 km west, and in 2018 the center of the hybrid zone retreated approximately 3.1 km east (Table 3). It is therefore possible that while we did not sample this transect prior to 2016, that a similar westward shift occurred along the Connecticut transect as we found along Massachusetts. For both transects, the mean population density of moths increases dramatically just east of the hybrid zone center in the region where winter moth is dominant (Figures 7 and 8). For the Massachusetts transect, the cline width varied from 9.62 to 69.42 km across years with a mean of 39.03 +- 7.01 km, and for the Connecticut transect, the cline width varied from 26.82 to 50.3 km across years with a mean of 40.18 +- 6.97 km (Table 3).

Comparison of minimum winter temperatures

There was a highly significant difference in the mean of the minimum winter temperatures observed during sample period between the two transects (t = -20.849, P < 0.001). For the Massachusetts transect, there was also a highly significant difference across the transect (t = 12.02, P < 0.001) with traps located in western Massachusetts having mean minimum winter temperatures near -24degC and those in eastern Massachusetts having mean minimum winter temperatures near -20degC (Figure 9). The sharp increase in temperatures in the eastern third of the Massachusetts transect (Figure 9) is associated with a sharp decline in landscape elevation between 75 to 100 km from T00. In contrast, there were no significant differences observed across the Connecticut transect (t = -0.773, P > 0.05), with traps located across the transect having minimum winter temperatures at or around -17 to -16degC, and all traps on this transect located at or near sea level.

Discussion:

Documenting the establishment and formation of new hybrid zones in real time is critical for understanding the spatial and temporal nature of these regions of genetic interchange (Abbott et al., 2013; Mallet, 2005). In addition, understanding the dynamics of hybridization between native and non-native species may be particularly important for understanding how invasive species become established and spread, because reproducing with a native species could alleviate Allee effects that limit the establishment of small populations due to stochastic disturbances and mate-finding (Ellstrand & Schierenbeck, 2000; Espeland, 2013; Mesgaran et al., 2016; Pfennig, Kelly, & Pierce, 2016; Yamaguchi, Yamanaka, & Liebhold, 2019). Here, we document the real time formation of a clinal (sensu Taylor et al., 2015) hybrid zone, following the introduction of the invasive winter moth to the northeastern United States. Our analyses suggest that the location of the center of this hybrid zone might not be regulated primarily by environmental variables, but appears to be behaving as a tension hybrid zone. Tension hybrid zones are characterized by their independence from en-

vironmental variables, narrow geographic width, low frequencies of hybridization, and with the geographic location determined by a balance between dispersal (dependent on population density) and selection against hybrids (Barton & Hewitt, 1985; Key, 1968; Smith, Hale, Kearney, Austin, & Melville, 2013). As shown by examining our two transects, the hybrid zone is narrow, with a mean of ˜ 40 km across all years in both transects, and hybridization rate is low (˜ 6 % in both transects). The location of the hybrid zone also appears to be dependent on population size, which would influence dispersal rate because the center of the hybrid zone is near the region where the population size of winter moth drops to where it is similar to the endemic Bruce spanworm populations (Figures 7 and 8). The final feature of a tension zone, low hybrid fitness, has also been demonstrated in this system. Laboratory rearing of winter moth and Bruce spanworm produced 93.5 and 94.1% viable eggs, respectively, while crosses between winter moth females and Bruce spanworm males produced 60.8% viable eggs and crosses between Bruce spanworm females and winter moth males produced just 22.1% viable eggs (Havill et al., 2017). The near complete lack of Bruce spanworm backcrosses also indicates low hybrid fitness in this system (Havill et al., 2017; Andersen et al., 2019; this study). Interestingly, these two species appear to have few pre-zygotic barriers to hybridization since they share the same sex pheromone (Elkinton et al., 2011) and have overlapping mating flight periods (Andersen, unpublished data). The barriers to hybridization in this system, therefore, appear to be almost entirely made up of post-zygotic incompatibilities resulting from > 500,000 years of allopatric divergence (based on an averaged observed mitochondrial percent divergence between these two species of 7.5% documented in Gwiazdowski, Elkinton, DeWaard, & Sremac, 2013; and the newly calibrated mitochondrial mutation rate of approximately 14.5% per million years for insects presented in Key, Frederick, & Schul, 2018).

Separating environmental factors (e.g., climate, land use, etc.) from population factors (e.g., dispersal, abundance, hybrid fitness, etc.), may not always be entirely feasible, and could, in part, explain why there is a paucity of documented examples of this type of hybrid zone between an introduced and a native species. However, by comparing our two transects that differed in extreme minimum temperatures (Massachusetts from -20degC to -24degC, and Connecticut ˜ -17degC), our results indicate that extreme minimum winter temperatures are not constraining the geographic location or width of the winter moth x Bruce spanworm hybrid zone. That said, it should be noted that researchers in Europe have observed that populations of winter moth can rapidly adapt to changes in environmental conditions (van Asch, Salis, Holleman, van Lith, & Visser, 2013), and as such the winter moth x Bruce spanworm hybrid zone presents an exciting system to study the combined roles of local adaptation and hybridization in the establishment an invasive species under changing climate regimes.

In contrast to direct environmental constraints on the location of the hybrid zone, we believe that population factors are more important for explaining the differences in relative population densities of these two species and therefore the stability and dynamics of the hybrid zone. One such constraint might be top-down pressure by natural enemies of both species. The biological control of winter moth in North America is one of the best-known examples of the successful use of importation biological control (Van Driesche et al., 2010) to reduce the ecological and economic impacts of a non-native forest defoliator with a broad host range (Elkinton et al., 2015; Embree, 1966; Kimberling et al., 1986; Roland & Embree, 1995). Recently, Elkinton et al. (2021) showed that the introduction of a single specialist natural enemy to the Northeast was able to convert winter moth to non-pest status. These introduced natural enemies have been incredibly effective at reducing the abundance of winter moth in high density locations, but at low densities, numerous authors have found that native pupal parasitoids play an important role in regulating winter moth population sizes (Frank, 1967a, 1967b; Horgan, 2005; Horgan & Myers, 2004; Latto & Hassell, 1987; Raymond et al., 2002; Roland, 1994; Roland & Embree, 1995, Broadley 2018). For example, in the Northeast, Broadley (2018) found that mortality caused by native generalist pupal parasitoids was lowest in the eastern coastal regions and increased as she sampled locations into the western interior portions of this region. Pupal parasitism could therefore play an important role in limiting the population sizes of both species, and as a result providing the necessary balance for a tension hybrid zone to exist in this system (see Taylor et al., 2015). It will be interesting to observe whether the location of the hybrid zone shifts east as the population density of winter moth continues to decrease due to the impacts of biological control. Indeed, the eastward retreat

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of the hybrid zone in 2018, the last year of our study (Figures 7 and 8), may indicate that this has begun.

It is commonly acknowledged that during the invasion process, the probability of establishment of non-native species can be influenced by native predators, parasitoids, competitors, and/or microbial communities through a process known as biotic resistance (Alpert, 2006; Dawkins & Esiobu, 2016; Kimbro, Cheng, & Grosholz, 2013; Levine, Adler, & Yelenik, 2004). For several decades there has been considerable concern expressed in the literature about the risk of hybridization between native and introduced species resulting in the "hybridization to extinction" of the native species (Allendorf et al., 2001; Ayres, Zaremba, & Strong, 2004; Hinton, 1975; Levin, 2002; Levin, Francisco-Ortega, & Jansen, 1996; Prentis, White, Radford, Lowe, & Clarke, 2007; Rhymer & Simberloff, 1996; Todesco et al., 2016; Wolf, Takebayashi, & Rieseberg, 2001). Under a tension hybrid zone model, however, the continued exchange of genetic material and the resulting production of low-fitness hybrids, could result in a reduction in the rate of spread of the introduced species by stabilizing the geographic center of the hybrid zone, creating what we believe is an underappreciated form of biotic resistance to invasion (*sensu*Levine et al., 2004). As such, we encourage additional research into the possible role of hybridization for limiting the establishment and spread of non-native species.

Conclusions

Here we present, to our knowledge, the first example of the real-time establishment of a clinal hybrid zone between a non-native invasive insect pest and a native species. This hybrid zone appears to be a tension hybrid zone, with hybrid individuals having reduced fitness compared to their parents, with the geographic center of the hybrid zone constrained by the population sizes of the two parent-species, rather than directly in relationship to an environmental or landscape variable. By examining two transects (separated by less than 125 km), we find evidence that the temporal and spatial dynamics of hybrid zones are complex, and we encourage further examination of the spatial and temporal dynamics of hybrid zones between native and non-native species so that comparative analyses can be conducted. Lastly, our results highlight the importance of long-term datasets for the study of evolutionary biology and invasion ecology, and we encourage future work to reexamine the movement and stability of this hybrid zone.

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Data Accessibility: Genotype scores for all samples are provided as a structure datafile in single-column format in the Supplemental files.

Author Contribution: All authors contributed to the study design and the preparation of the manuscript. JCA and NPH analyzed the genetic datasets. JLC analyzed the climate dataset. GHB and JLC collected field samples. AC and JSE provided laboratory access.

Tables and Figures:

Table 1: Number of moths classified as winter moth, Bruce spanworm, F1 hybrids ("F1"), F2 hybrids ("F2"), winter moth-backcross, or Bruce spanworm-backcross for each surveyed transect year from 2007-2018 in Massachusetts using NewHybrids.

Year	Winter Moth	Bruce Spanworm	F1	F2	Winter Moth-Backcross	Bruce Spanworm-Backcross
2007	110	227	12	0	3	0
2008	164	195	7	0	1	0
2009	298	132	28	2	14	0
2010	85	223	7	3	7	0
2011	147	240	29	1	0	0
2012	129	203	20	1	3	0
2013	154	167	17	0	2	0
2014	133	178	25	2	4	0
2015	177	123	18	1	0	1
2016	181	182	8	0	7	0
2017	326	975	15	14	29	0
2018	257	527	24	1	2	0

Table 2: Number of moths classified from 216-2018 in Connecticut as per Table 1.

Year	Winter Moth	Bruce spanworm	F1	F2	Winter Moth-Backcross	Bruce spanworm- backcross
2016	549	746	44	9	6	0
2017	793	681	14	103	7	0
2018	514	625	13	18	1	0

Table 3: Best-fit cline model and geographic location of the cline center of the winter moth x Bruce spanworm hybrid zone for the Massachusetts transect for each year from 2007-2018, and Connecticut transect for 2016-2018, estimated using the R package 'hzar'. The confidence interval two log likelihood units above and below the center and width values are in parentheses. Cline centers and widths for 2009, 2011, and 2012 could not be estimated due to failures in model conversion.

Transect	Year	Model	Cline Center (km)	Cline Width (km)
Massachusetts	2007	Fixed frequencies, no tails	130.64 (129.05, 132.18)	14.02 (10.83, 18.33)
Massachusetts	2008	Free frequencies, no tails	127.56 (126.05, 128.95)	9.87 (6.62, 14.16)
Massachusetts	2009	Free frequencies, no tails	N/A	N/A
Massachusetts	2010	Fixed frequencies, no tails	115.23 (109.97, 121.12)	69.42 (53.42, 92.55)
Massachusetts	2011	Free frequencies, no tails	N/A	N/A
Massachusetts	2012	Free frequencies, no tails	N/A	N/A
Massachusetts	2013	Free frequencies, with tails	107.13 (103.42, 110.49)	49.07 (39.11, 63.1)
Massachusetts	2014	Fixed frequencies, no tails	104.66 (100.41, 108.57)	53.66 (42.19, 68.94)
Massachusetts	2015	Fixed frequencies, no tails	102.44 (98.79, 105.68)	42.84 (34.17, 54.8)
Massachusetts	2016	Fixed frequencies, no tails	98.64 (94.24, 102.52)	47.57 (38.02, 60.16)
Massachusetts	2017	Free frequencies, no tails	95.84 (93.03, 97.21)	9.62 (5.89, 21.3)
Massachusetts	2018	Fixed frequencies, no tails	103.58 (100.4, 106.66)	55.17 (47.25, 64.7)
Connecticut	2016	Fixed frequencies, no tails	109.23 (106.75, 111.97	43.41 (37.25, 49.11)
Connecticut	2017	Fixed frequencies, no tails	100.31 (97.22, 103.19)	50.3 (40.56, 58.34)
Connecticut	2018	Fixed frequencies, no tails	103.42 (101.01, 105.77)	26.82 (21.85, 32.84)

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Figure 1. Locations of survey traps along the Massachusetts transect (blue) and the Connecticut transect (yellow). The western and eastern most points along each transect are labeled for reference, as are the traps most proximal to the center of the hybrid zone in 2018.

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Figure 2. Proportional assignment of individuals collected along the Massachusetts transect as either pure winter moth (black), pure Bruce spanworm (white), or to one of four hybrid categories (grey) in NewHybrids.

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Figure 3. Proportional assignment of individuals collected along the Connecticut transect as either pure winter moth (black), pure Bruce spanworm (white), or to one of four hybrid categories (grey) in NewHybrids (A). The number of genotyped individuals classified as F1 hybrids (red), F2 hybrids (orange), or winter moth backcrosses (blue) are presented in part B.

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Figure 4. The number of genotyped individuals classified as F1 hybrids (red), F2 hybrids (orange), or winter moth backcrosses (blue) as calculated in NewHybrids.
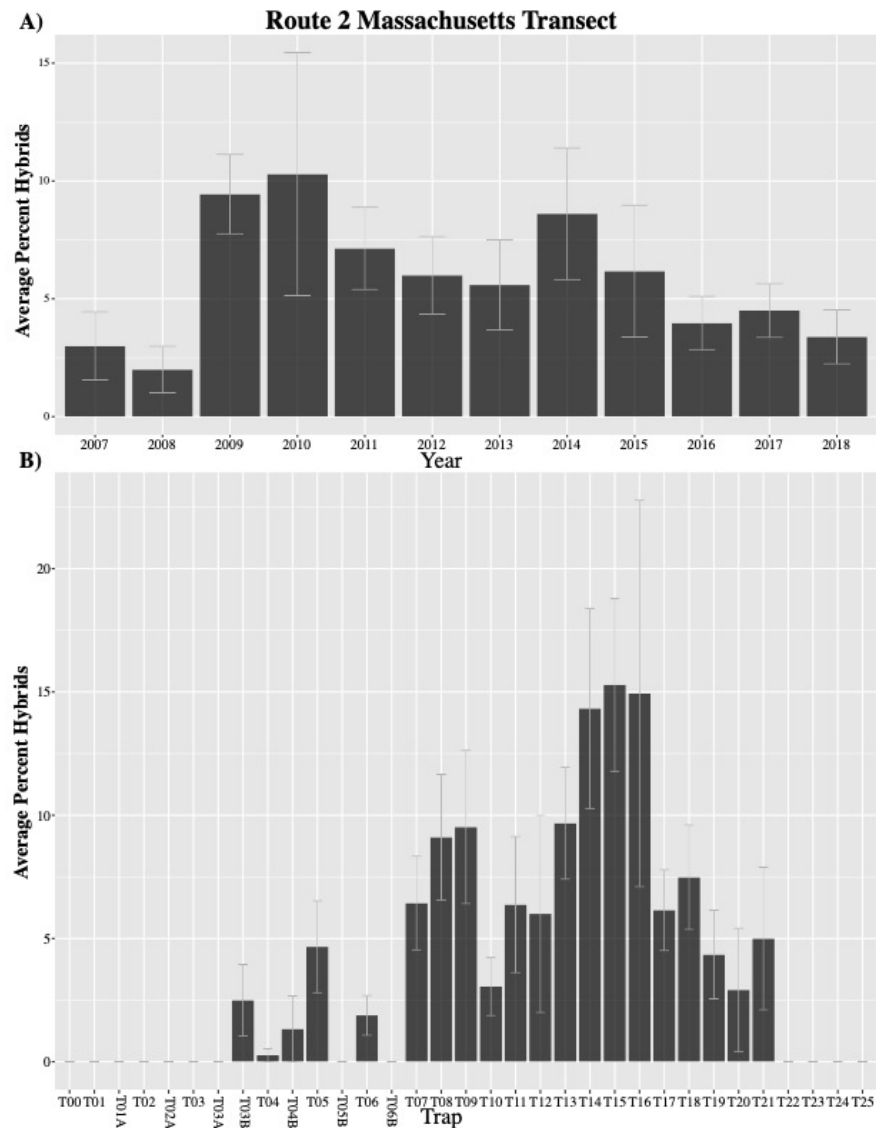

Figure 5. The average percentage (± SE) of genotyped individuals classified as hybrids in 2007-2018 (A), and the average percentage (± SE) of genotyped individuals classified as hybrids at each trap (B) for all
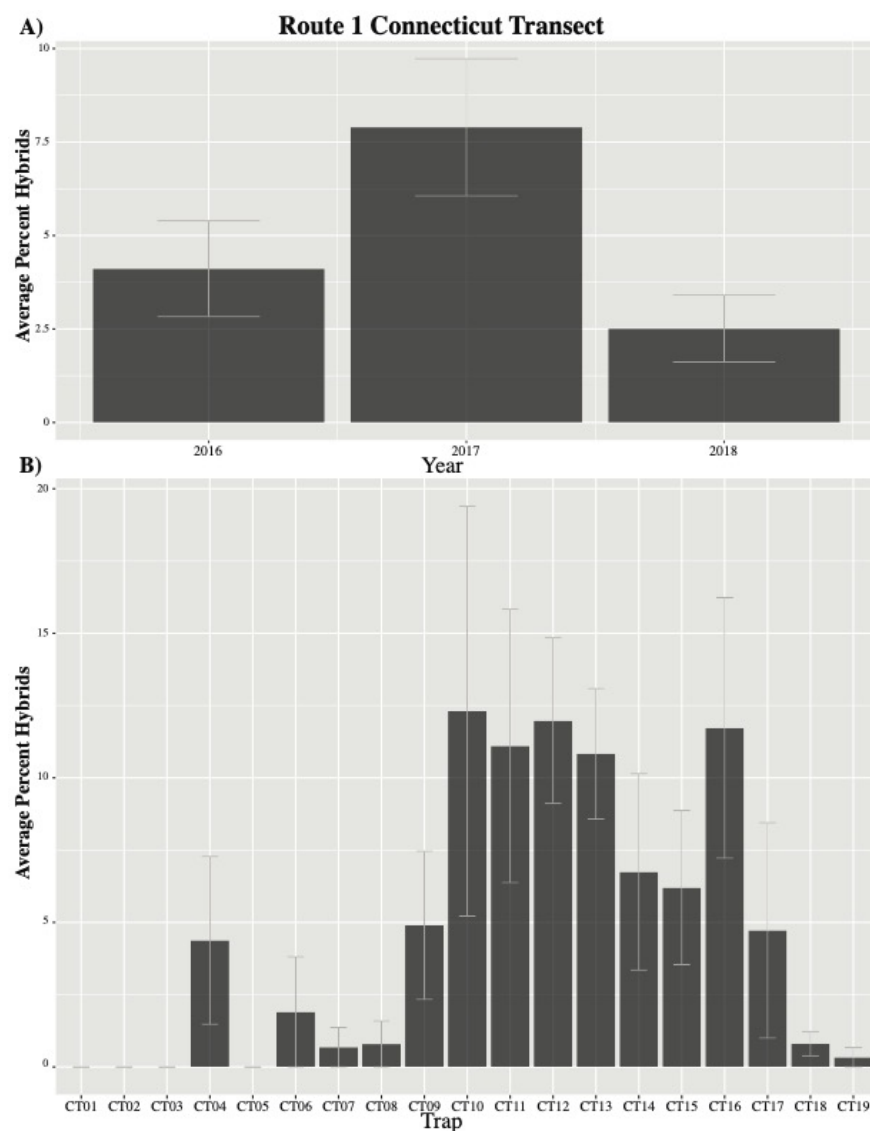
15

years across the Massachusetts transect.

Figure 6. The average percentage (± SE) of genotyped individuals classified as hybrids in 2016-2018 (A), and the average percentage (± SE) of genotyped individuals classified as hybrids at each trap (B) for all years across the Connecticut transect.
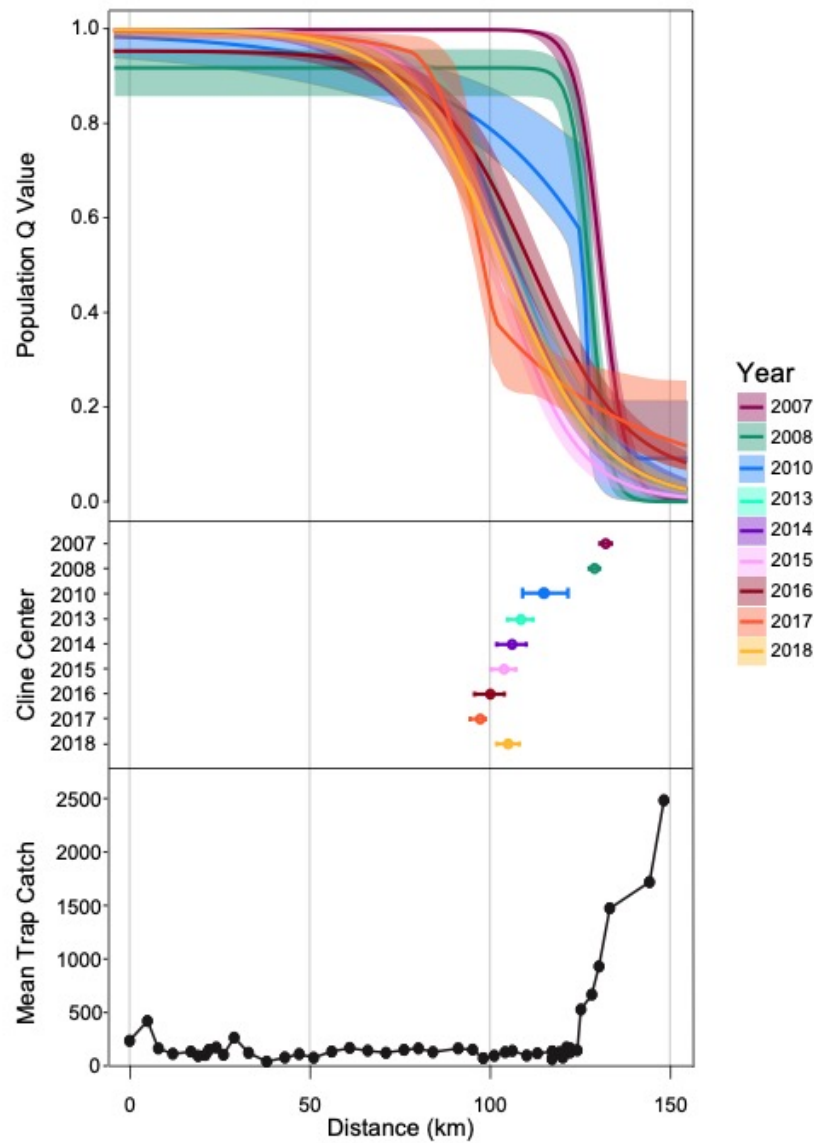
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Figure 7. Cline analysis showing the fit of the best model based on the proportional assignment of populations to the Bruce spanworm genetic cluster (Q) for each sampled year along the Massachusetts transect (top) and the geographic center $\pm$ 2LL as estimated in the R package 'hzar' (middle). The number of individuals collected at each trap averaged across the 2007-2018 sample years (bottom). Distances are presented in relationship to T00.
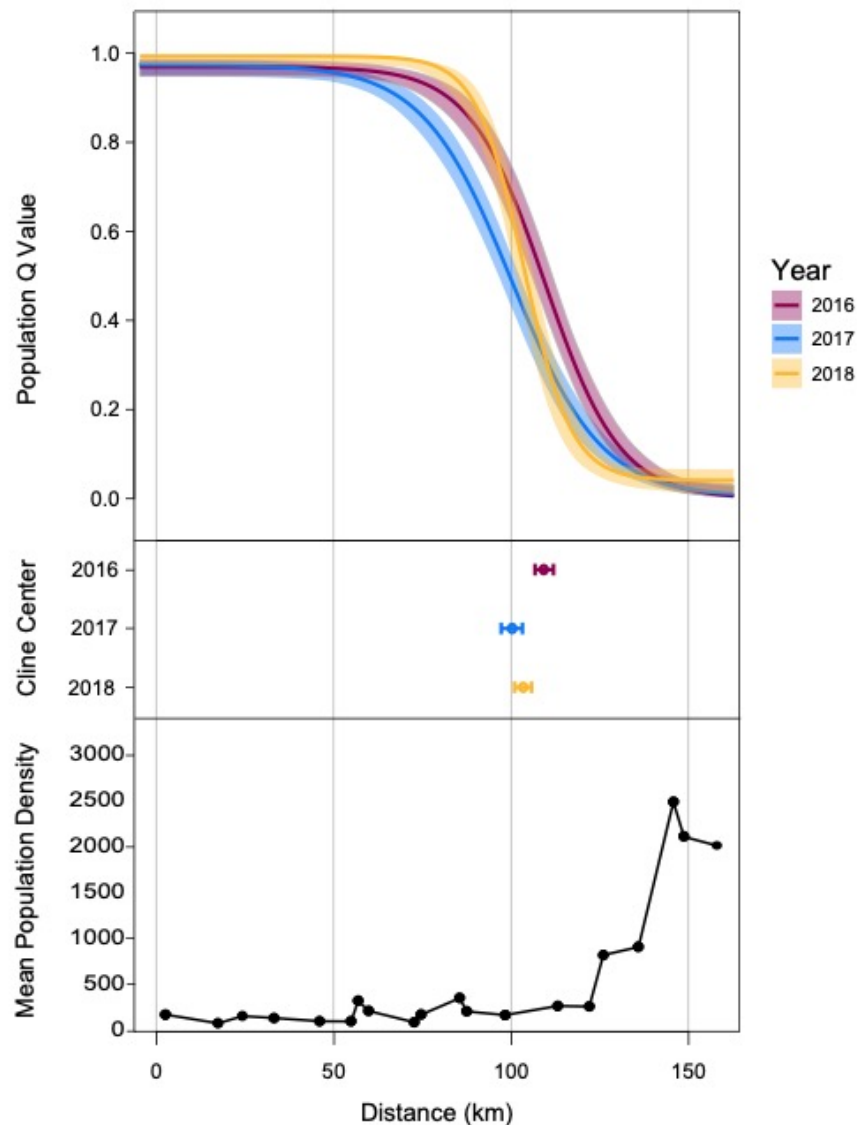
Figure 8. Cline analysis showing the fit of the best model based on the proportional assignment of populations to the Bruce spanworm genetic cluster (Q) for each sampled year along the Connecticut transect (top) and the geographic center $\pm$ 2LL as estimated in the R package 'hzar' (middle). The number of individuals collected at each trap averaged across the 2016-2018 sample years (bottom). Distances are presented in relationship to CT01.

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image9.emf available at https://authorea.com/users/388368/articles/539000-real-time-geographic-settling-of-a-hybrid-zone-between-the-invasive-winter-moth-operophtera-brumata-l-and-the-native-bruce-spanworm-o-bruceata-hulst

Figure 9. Mean absolute minimum temperatures as extracted from the PRISM dataset for trap locations along the Massachusetts transect (dashed line) and the Connecticut transect (solid line) from December 1st through March 31st for 2007-2018. Distances are presented in relationship to T00 for the Massachusetts

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transect, and CT01 for the Connecticut transect.

Route 2 Massachusetts Transect

A) **Route 1 Connecticut Transect - Proportional Assignments**

B) **Route 1 Connecticut Transect - Hybrid Classes**

Route 2 Massachusetts Transect

A)

Route 2 Massachusetts Transect

B)

A) **Route 1 Connecticut Transect**

B)